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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT
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INVENTOR(S)/APPLICANT(S)			
LAST NAME	FIRST NAME	MI	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY
ARKINSTALL	Stephen	J	Belmont, MA
ESHKOL	Aliza		La Rippe, Switzerland
MACNAMEE	Michael	C	Cambridge, UK
MCKENNA	Sean	D	Duxbury, MA
PALMER	Stephen	S	Plympton, MA

[ ] Additional inventors are being named on separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

INHIBITORS OF PDE ENZYMES IN INFERTILITY

CORRESPONDENCE ADDRESS

Direct all correspondence to the address associated with **Customer Number 001444**, which is presently:

BROWDY AND NEIMARK, P.L.L.C.
624 Ninth Street, N.W., Suite 300
Washington, D.C. 20001-5303

ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification	Number of Pages	25	[ ] Applicant claims small entity status. See 37 C.F.R. §1.27
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The invention was made by an agency of the United Stated Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.

By: _____
Roger L. Browdy
Registration No.: 25,618

Date: April 1, 2003

Inhibitors of PDE enzymes in infertility

Field of Invention

The invention relates to the field of ovulation induction (OI) and assisted reproductive technologies (ART), such as controlled ovarian hyperstimulation (COH) for *in vitro* fertilisation (IVF), using PDE inhibitors for stimulation of follicular growth and development.

Background of the Invention

Ovulation induction is the treatment of anovulatory or ammenorheic women to cause the release of a single oocyte into the fallopian tubes for *in vivo* fertilisation or intrauterine insemination (IUI). In a conventional regimen for OI, a patient is treated with follicle-stimulating hormone (FSH), usually at about 150 IU FSH per day, starting on about day three after spontaneous or induced menstruation. The administration of FSH causes the development and growth of follicles in the ovary. As the follicles develop, one follicle, the dcminant follicle, will surpass the others in its response to FSH, while the other follicles will undergo atresia. The goal of an OI regimen is to cause a single oocyte to be released, so as to avoid multiple pregnancies. FSH administration is continued until there is one follicle of mean diameter greater than or equal to about 16-18 mm (follicles can be evaluated by ultrasound). The period in which FSH is administered is called the stimulatory phase. At this point a relatively large dose (5'000-10'000 IU) of human chorionic gonadotrophin (hCG) is administered to mimic the natural luteinising hormone (LH) peak or surge that occurs mid-cycle, to trigger release of a single oocyte into the fallopian tubes. The administration of the ovulation-triggering dose of hCG (or other agent having LH-activity) marks the end of the stimulatory phase. The patient is instructed to have intercourse 24 to 38 hours after administration of the large dose of hCG.

In some patients undergoing OI it may be desirable to suppress pituitary gonadotrophins by administering a gonadotrophin releasing hormone (GnRH) agonist prior to commencing therapy with FSH. Administration of a GnRH agonist is started in the luteal phase of a menstrual cycle (usually on about day 20 of a menstrual cycle). Suppression

of ovarian function usually takes from 8 to 21 days with a GnRH agonist, and may be monitored by monitoring LH or oestradiol levels (LH < 5 IU/L, E_2 < 50 pg/ml generally indicate adequate suppression). The stimulatory phase is then started by administration of FSH. The use of a GnRH agonist suppresses the natural LH peak or surge which can trigger the release of oocytes prematurely. This allows better timing of release of the oocyte, and consequently intercourse. In patients suffering from polycystic ovarian syndrome (PCOS), it is also desirable to use a GnRH agonist, because these patients often have inappropriately high endogenous LH levels, it also permits the suppression of LH throughout the stimulatory phase, permitting better response to FSH.

OI is also often carried out using agents that provoke endogenous FSH release, such as clomiphene citrate or aromatase inhibitors. Clomiphene citrate is administered during a stimulatory phase (usually a dose of 50 to 100 mg on days 3 to 7 or 5 to 9 of the menstrual cycle), causing an increase in endogenous FSH secretion, leading to follicular growth. Aromatase inhibitors, for example Letrozole, Anastrozole, YM-511, may be given on days 3 to 7 or 5 to 9 of the menstrual cycle, and also provoke a release of endogenous FSH, as described in WO 02/083239.

In contrast to OI, where a single ovulatory follicle and a single oocyte is desired, in assisted reproductive technologies (ART) regimens, it is desired to collect as many oocytes in a single cycle as possible. Treatment of infertility by ART, such as *in vitro* fertilisation (IVF), intracytoplasmic sperm injection (ICSI), Gamete Intrafallopian Transfer Procedure (GIFT), and Zygote Intrafallopian Transfer Procedure (ZIFT), requires controlled ovarian hyperstimulation (COH) to increase the number of female gametes[1]. Standard regimens for COH in ART include a down-regulation phase in which endogenous LH is suppressed by administration of a GnRH agonist starting in the luteal phase of a menstrual cycle (usually on about day 20 of a menstrual cycle). Suppression of ovarian function usually takes from 8 to 21 days with a GnRH agonist, and may be monitored by monitoring LH or oestradiol levels (LH < 5 IU/L, E_2 < 50 pg/ml generally indicate adequate suppression). Down regulation is followed by a stimulatory phase in which follicular development is induced by daily administration of follicle stimulating hormone (FSH), usually at about 75-600 IU/day.

Alternatively, a GnRH antagonist may be used, instead of a GnRH agonist, in which case follicular stimulation with FSH is started, usually on day 1, 2 or 3 after spontaneous or induced menstruation, and endogenous LH production is suppressed by administration of a GnRH-antagonist starting on about day 6 after menses. GnRH antagonist and FSH administration are continued until the criteria for administration of an ovulation-triggering dose of hCG are met, as described below.

In COH protocols for ART, multiple follicular development is the desired aim. During the stimulatory phase, the ovaries are examined by ultrasound, to detect and measure the developing follicles. When there are at least 3 follicles with a mean diameter greater than 16 mm (preferably one of 18 mm), an injection of hCG (5,000-10,000 IU) is given to trigger ovulation. Oocyte recovery is timed for 36-38 hours after the hCG injection. Oocytes are usually recovered from pre-ovulatory follicles, by aspiration.

As described above, both ovulation induction (OI) and assisted reproductive technologies (ART) regimens comprise two main phases: a stimulatory phase and an ovulatory phase. The stimulatory phase starts with the first administration of an agent having follicle stimulating activity (such as FSH), and is usually 6 to 10 days long. During the stimulatory phase, follicle-stimulating hormone (FSH), or an agent exerting follicle-stimulating activity, or stimulating endogenous FSH release, stimulates ovarian follicular growth. Administration of FSH or an agent exerting FSH-like activity or an agent stimulating endogenous FSH release need not be continuous in the stimulatory phase, nor need it continue until the end of the stimulatory phase. The beginning of the ovulatory phase marks the end of the stimulatory phase. The ovulatory phase begins with the administration of a large amount of an agent having LH-activity (such as 5'000-10'000 hCG). This causes final maturation of the oocytes and triggers ovulation through rupture of the follicles. Alternative agents that can act in either the stimulatory phase or the ovulatory phase are desirable, particularly orally available agents. US 2002/0103106 A1 (Palmer *et al.*) discloses the oral and subcutaneous use of inhibitors of type IV phosphodiesterase in the ovulatory phase, to trigger ovulation. US 2003/0018037 (Westbrook Lempriere *et al.*) discloses the use of PDE5 inhibitors after ovulation in a non-assisted cycle to improve embryo survival, increase birth weight, increase uterine blood flow and increase progesterone serum levels.

Treatment with FSH for either OI or ART involves daily injections of relatively large doses of FSH (75-600 IU FSH daily) during the stimulatory phase. The daily injections can cause patient discomfort and inconvenience and can be relatively costly. In addition, large doses of FSH can cause ovarian hyperstimulation syndrome (OHSS), a potentially life-threatening condition. Replacement of FSH with alternative medicaments having the ability to aid follicular growth and which avoid the risks of OHSS would be highly desirable, particularly. Furthermore, the provision of a preparation which acts with FSH to cause follicular growth would also be highly desirable, as this could augment low endogenous FSH levels, to cause follicular growth in those patients who are anovulatory due to low endogenous FSH levels, or to augment exogenously administered FSH, permitting an improved response in poor responders in ART, or permitting the same response in ART but with lower dose and/or less frequent injections of FSH, and at the same time avoiding the risks of OHSS.

Summary of the invention

It is an object of the invention to provide an improved method of OI.

It is a further object of the invention to provide an improved method of COH for ART.

In a first aspect, the invention provides a use of an inhibitor of a phosphodiesterase (PDE) enzyme for the preparation of a medicament for stimulating ovarian follicular growth in a patient.

In a second aspect, the invention provides a use of an inhibitor of a phosphodiesterase (PDE) enzyme for stimulating ovarian follicular growth in a patient.

In a third aspect the invention provides a method for stimulating ovarian follicular growth in a patient in need thereof, comprising administering an effective dose of a phosphodiesterase inhibitor to the patient.

Detailed description of the invention

Brief description of the drawings

Figure 1 shows the number of oocytes ovulated per rat in immature female rats treated with a suboptimal dose of FSH ("Low FSH":151.5 ng X 4 per rat) and rats treated with the suboptimal dose of FSH ("Low FSH") plus Dipyridamole (1, 5, 25 mg/kg x 4 per rat).

Figure 2 shows the number of oocytes ovulated per rat in immature female rats treated with a suboptimal dose of FSH ("Low FSH"; 151.5 ng X 4 per rat) and rats treated with the suboptimal dose of FSH ("Low FSH") plus Zaprinast (1, 5, 25 mg/kg x 4 per rat).

Figure 3 shows the number of oocytes ovulated per rat in immature female rats treated with a suboptimal dose of FSH ("Low FSH"; 151.5 ng X 4 per rat) and rats treated with the suboptimal dose of FSH ("Low FSH") plus Sildenafil (1, 5, 25 mg/kg x 4 per rat).

Figure 4 shows the number of secondary follicles in rats treated with low dose FSH ("Low FSH"; 151.5 ng X 3 per rat), high dose FSH ("High FSH"; 606.2 ng X 3 per rat), and low dose FSH plus Sildenafil (25 mg/kg x 3 per rat).

Figure 5 shows the number of antral follicles in rats treated with low dose FSH ("Low FSH"; 151.5 ng X 3 per rat), high dose FSH ("High FSH"; 606.2 ng X 3 per rat), and low dose FSH plus 75 mg/kg Sildenafil.

Figure 6 shows the number of oocytes ovulated per rat in immature female rats treated with low dose FSH ("low FSH"; 151.5 ng X 3 per rat), high dose FSH ("High FSH"; 606.2 ng X 3 per rat), and low dose of FSH (151.5 ng X 4 per rat) plus Sildenafil (1, 5, 25 mg/kg x 4 per rat) administered in an aqueous buffer.

The inventors have surprisingly found that molecules that are inhibitors of phosphodiesterase enzymes (PDE's), preferably PDE types selected from 1, 5, 6, 7, 9, 10, 11, more preferably PDE types 1 and 5, are capable of aiding follicular growth in the presence of sub-optimal amounts of FSH. In a preferred embodiment, the inhibitor is selective for phosphodiesterase types 1 and/or 5 (a "selective" inhibitor of PDE types 1 and/or 5, for example, means a molecule exhibiting an IC50 for PDE types 1 and/or 5 that is at or about 10-fold, preferably 100-fold, more preferably 1000-fold, lower than the IC_{50} of the molecule for other PDE types. The IC50 of a molecule for a PDE enzyme can be measured by an in vitro assay, as reported, for example in Thompson *et al.*; *Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme*; *Adv. Cyclic Nucleotide Res.*; **1979**, *10*, 69-92). In a further preferred embodiment, the PDE inhibitor is an inhibitor of PDE type 1, most preferably a selective

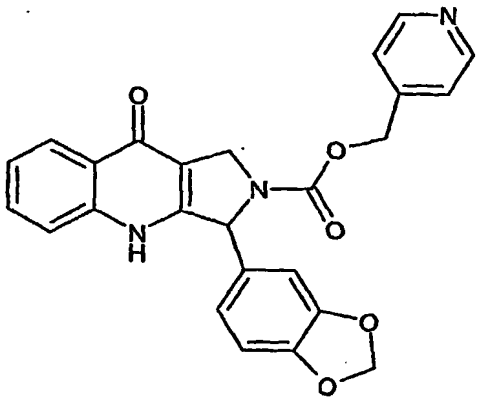
inhibitor of PDE type 1. Preferably the PDE inhibitor is a non-peptide PDE inhibitor. Also preferably the PDE inhibitor has a molecular weight under 500 Da.

The PDE inhibitor is administered starting in the stimulatory phase, before ovulation, and is preferably stopped before or on the day when the ovulatory phase is started by administration of large dose of an agent having LH-activity (such as 5'000-10'000 hCG). Most preferably administration of the PDE inhibitor stops two, one or zero days before the day on which hCG is administered. Most preferably administration of the PDE inhibitor stops on the day on which hCG is administered.

Examples of preferred PDE inhibitors include:

5-[2-ethoxy-5-(4-methyl-1 -piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil); Zaprinast; dipyrimadole; 5-(2-ethoxy-5-morpholinoacetylphenyl)-1 -methyl-3-n-propyl-1,6-dihydro-7H-20 pyrazolo[4,3-d]pyrimidin-7-one; 3-ethyl-5-[5-(4-ethylpiperazin- 1 -ylsulphonyl)-2-n-propoxyphenyl]-2-(pyr- idin-2-yl) methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridi- n-3-yl]-2-(pyridin-2-yl) methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; (+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7 H-pyrazolo[4 ,3-d] pyrimidin-7-one; 5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-[2-iso-butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-[2-ethoxy-5-(4-ethylpiperazin-1 -ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-(5-acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1 -isopropyl-3-azetidinyl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2 ,6-dihydro-7H-pyrazolo [4,3-d]pyrimidin-7-one; (6R, 12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(- 3,4-methylenedioxyphenyl)pyrazino[2',1 ':6,1]pyrido[3,4-b]indole-1,4-dione (Tadalafil; IC-351), the compound of examples 78 and 95 of published international application W0 95/19978, as well as the compound of examples 1, 3, 7 and 8 therein; 2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo [5,1 -f][1,2,4]triazin-4-one (vardenafil); the compound of example 11 of published international application W093/07124 (EISAI); compounds 3 and 14 from

Rotella D P, J. Med. Chem., 2000, 43,1257; 4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2H)pyridazinone; 1 -[4-[(1,3-benzodioxol-5-ylmethyl)amiono]-6-chloro-2-quinozolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-cis-5,6a,7,9,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-[4,5]imidazo[2,1-b]purin-4(3H)one; furaziocillin; cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a-octahydrocyclopent[4,5]-imidazo[2- ,1 -b]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6- carboxylate; 3-acetyl-1 -(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl)propoxy)-3-(2H) pyridazinone; 1-methyl-5(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro- 7H-pyrazolo (4,3-d)pyrimidin-7-one; 1 -[4-[(1,3-benzodioxol-5-yl methyl)amino]-6-chloro-2-quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt; Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; see WO 96/26940); Pharmaprojects No. 5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 and E-4010 (Eisai); Bay-38-3045 & Bay-38-9456 (Bayer); Vinpocetine (Richter Gideon); SCH-51866 (Schering-Plough), SCH-59498, compounds no. 31, 33, 50 described in Ahn *et al.*; *J. Med. Chem.*; **1997**, *40*, 2196-2210, dipyridamole, AWD-12-171 and AWD-12-217 (ASTA Medica), BMS-341400 (Bristol Meyers Squibb), UK-343,664 (Pfizer), 5E-3623, 5E-3569, 5E-3657, E4021 (Eisai), KS-505a (Kyowa Hakko Kogyo), YC-1 (Yung Shin Pharmaceutical Industries), IDDB reference number 323951 (Bayer), WIN-61691 (Sanofi Winthrop), FR226807 (Fujisawa), IDDB references 461317, 462503, 461321, 461324, 466146 (Johnson & Johnson),



compounds listed in Table 1 of Jiang *et al.*; *J. Med. Chem.*; **2003**, *46*, 441-444, particularly compounds 20b, 20e, 20f, 20l, 20o, 20p, (-)-20q, 20t, 20u, 20v, 20w and 26a.

Particularly preferred PDE inhibitors are Sildenafil and Zaprinast, Dipyrimidole, and compounds no. 31 and 33, described in Ahn *et al.*; *J. Med. Chem.*; **1997**, *40*, 2196-2210.

The PDE inhibitor is preferably administered to the patient during a stimulatory phase of from at or about two to at or about ten days, more preferably at or about three to at or about eight days, preferably starting on at or about day 2 or day 3 of the menstrual cycle. For OI, administration is preferably continued until there is one follicle of mean diameter greater than or equal to about 16-18 mm (and preferably not more than two follicles of mean diameter greater than 14 mm). Administration of the PDE inhibitor preferably stops on the day that hCG is administered to begin the ovulatory phase.

For ovulation induction, the PDE inhibitor may be administered alone (acting with endogenous FSH), or it may be administered in conjunction with FSH or an agent having FSH activity, or stimulating endogenous FSH release. The administration of the PDE inhibitor in conjunction with another follicle stimulating agent may be simultaneous, separate or sequential. Agents having FSH activity include recombinant and urinary FSH, and also FSH mixed with varying amounts of LH, such as hMG. Agents having FSH activity also include analogues of FSH, for example, CTP-FSH [a long-acting modified recombinant FSH, consisting of the wild type α-subunit and a hybrid β-subunit in which the carboxy terminal peptide of hCG has been fused to the C-terminal of the β-subunit of FSH, see LaPolt *et al.*; Endocrinology; **1992**, *131*, 2514-2520; or Klein et al.; Development and characterization of a long-acting recombinant hFSH agonist; Human Reprod. 2003, 18, 50-56]. Also included is single-chain FSH-CTP, a single chain molecule, consisting of the following sequences (from N-terminal to C-terminal):

βFSH	βhCG-CTP(113-145)	αFSH

wherein βFSH signifies the β-subunit of FSH, βhCG CTP (113-145) signifies the carboxy terminal peptide of hCG and αFSH signifies the α-subunit of FSH, as described by Klein *et al.*[2] Agents which stimulate or lead to endogenous FSH production or release include

clomiphene citrate and aromatase inhibitors, such as, for example, Letrozole, YM-511, Anastrozole or Fadrozole [the use of these molecules in OI is disclosed, for example, in WO 02/083241 and WO 02/083239].

In a preferred regimen for OI, the patient is administered a PDE inhibitor, preferably a PDE 1 and/or 5 inhibitor, more preferably a selective PDE 1 and/or 5 inhibitor, most preferably a PDE 1 inhibitor, and most particularly preferably a selective PDE 1 inhibitor, preferably starting at or about day three after menstruation. This marks the beginning of the stimulatory phase. Administration of PDE inhibitor is continued, preferably on a daily basis, but may also be twice daily or on alternate days, or even in a single dose. The progress of developing follicles may be monitored by ultrasound. Follicular growth is judged to be sufficient when there is one follicle of mean diameter greater than or equal to at or about 16-18 mm, and preferably not more than two follicles with mean diameter greater than 16 mm, an ovulation trigger is given (marking the end of the stimulatory phase and the beginning of the ovulatory phase), using an agent having LH-activity, for example hCG (usually 5'000 to 10'000 IU), LH (25'000 to 70'000 IU) or a PDE IV inhibitor (as described in published US patent application no. 2002/0103106 A1). The patient is then instructed to have intercourse 24 to 36 hours after administration of the ovulation trigger. Alternatively fertilisation may be by intrauterine insemination (IUI).

In patients having non-stimulated and non-down-regulated (natural cycle) serum FSH levels below at or about 2 IU/litre (measured at or about day 2 after menstruation), ovulation induction should preferably be carried out using a PDE inhibitor in conjunction with FSH or agents having FSH activity, or agents stimulating an endogenous release of FSH. The dose of FSH required will be less than that required in patients who do not receive a PDE inhibitor to achieve the same or better response, in terms of number of follicles having a mean diameter of at or about 16 mm or greater on day 8 of stimulation.

When FSH or an agent having follicle-stimulating activity, or agents stimulating an endogenous release of FSH are used in OI, administration of FSH may be started before or after administration of the PDE inhibitor, or the two agents may be administered starting simultaneously. Preferably administration of FSH starts simultaneously with administration of the PDE inhibitor, or at or about one day before administration of the PDE inhibitor. The stimulatory phase begins with the first administration of FSH or PDE

inhibitor, whichever is first, or if only a PDE inhibitor is used, on the day that administration begins.

For controlled ovarian hyperstimulation (COH) for ART, a PDE inhibitor can be used without exogenous FSH (or an agent having follicle-stimulating activity), according to the invention, to enhance endogenous FSH levels, resulting in controlled ovarian hyperstimulation, and the development of multiple ovulatory follicles. If the patient has non-stimulated and non-down-regulated (natural cycle) serum FSH levels below at or about 5 IU/litre (measured at or about day 2 after menstruation), COH should preferably be carried out using a PDE inhibitor in conjunction with FSH or an agent having follicle-stimulating activity, or agents stimulating an endogenous release of FSH, such as those mentioned above. When FSH or an agent having follicle-stimulating activity, or agents stimulating an endogenous release of FSH are used in COH, administration of FSH may be started before or after administration of the PDE inhibitor, or the two agents may be administered starting simultaneously. Preferably administration of FSH starts simultaneously with administration of the PDE inhibitor, or at or about one day before administration of the PDE inhibitor. The stimulatory phase begins with the first administration of FSH or PDE inhibitor, whichever is first, or if only a PDE inhibitor is used, on the day that administration begins.

In a preferred regimen for COH, the patient is first down-regulated to suppress endogenous luteinising hormone (LH), by administration of a gonadotrophin releasing hormone (GnRH) agonist starting in the luteal phase of a menstrual cycle (usually on about day 20 of a menstrual cycle). Suppression of ovarian function usually takes from 8 to 21 days with a GnRH agonist, and may be monitored by monitoring LH or oestradiol levels (LH < 5 IU/L, E_2 < 50 pg/ml generally indicate adequate suppression). Down-regulation is followed by a stimulatory phase in which follicular development is stimulated using a PDE inhibitor, preferably a PDE 1 and/or 5 inhibitor, more preferably a selective PDE 1 and/or 5 inhibitor, more preferably a PDE 1 inhibitor, most preferably a selective PDE 1 inhibitor. The PDE inhibitor may be used alone or in conjunction with administration of follicle stimulating hormone (FSH) or an agent having FSH activity or an agent stimulating endogenous FSH release. The day on which stimulation is started with a PDE inhibitor or FSH, whichever is started first, is defined as the start of the stimulatory phase. The use of a PDE inhibitor permits the use of lower doses of FSH (or

equivalents) than would be used in the same patient if FSH alone were used for COH, while achieving the same or better follicular response, in terms of number of follicles having a mean diameter of at or about 16 mm or greater on day 8 of stimulation. Preferably the dose of FSH used when a PDE inhibitor is administered will be less than or equal to at or about 75% of the dose of FSH that would be required in the same patient without the PDE inhibitor, in order to achieve the same follicular response, more preferably the dose of FSH will be less than or equal to at or about 50% of the dose of FSH that would be required in the same patient without the PDE inhibitor, most preferably the dose of FSH will be less than or equal to at or about 30% of the dose of FSH that would be required in the same patient without the PDE inhibitor.

When a PDE inhibitor is administered, the dose of FSH will preferably be at or about 25-600 IU FSH daily, more preferably at or about 50-450 IU daily, most preferably 50-300 IU daily. Stimulation with PDE inhibitor in the absence or presence of exogenous FSH (or equivalents) is preferably continued until follicular growth is judged to be sufficient, i.e. when there are at least 3 follicles with a mean diameter greater than at or about 16 mm (preferably one of 18 mm), while continuing to administer the GnRH antagonist. An ovulation-triggering dose of an agent having LH-activity (e.g. 5'000- 10'000 IU of hCG) is then administered, as described for OI. Oocyte recovery is timed for 36-38 hours after the ovulation trigger. Oocytes are usually recovered from pre-ovulatory follicles, by aspiration. Oocytes are graded, fertilised *in vitro*, and embryos are selected for transfer to the uterus approximately 72-96 hours after collection.

GnRH agonists include, for example, Buserelin, Goserelin, Leuprorelin, Triptorelin and Nafarelin.

In another preferred regimen for COH, a GnRH antagonist is used. Without the administration of a GnRH agonist, follicular stimulation is started, usually on day 1, 2 or 3 after spontaneous or induced menstruation, with a PDE inhibitor, preferably a PDE 1 and/or 5 inhibitor, more preferably a selective PDE 1 and/or 5 inhibitor, more preferably a PDE 1 inhibitor, most preferably a selective PDE 1 inhibitor, in conjunction with administration of follicle stimulating hormone (FSH) or an agent having FSH activity or an agent stimulating endogenous FSH release. Then a GnRH-antagonist is administered starting on about day 6 after menses. Stimulation with PDE inhibitor and

FSH (or equivalents) is continued until there are at least 3 follicles with a mean diameter greater than 16 mm (preferably one of 18 mm). An ovulation triggering dose of an agent having LH-activity is then administered, as described for OI. The GnRH antagonist is administered up until the day of ovulation triggering.

In an alternate preferred regimen for COH, follicular stimulation is started by administration of an aromatase inhibitor, preferably on days 3 to 6 after menstruation, in conjunction with a PDE inhibitor, preferably a PDE 1 and/or 5 inhibitor, more preferably a selective PDE 1 and/or 5 inhibitor, more preferably a PDE 1 inhibitor, most preferably a selective PDE 1 inhibitor. On or about day 6, a GnRH antagonist is given, the aromatase inhibitor is stopped and injections of FSH are started. PDE inhibitor, FSH and GnRH antagonist administration are continued until the ovulation-triggering dose of hCG is administered.

GnRH antagonists include for example, Cetrorelix, Nal-Glu, Antide, Ganirelix, Azaline B and Antarelix.

Pharmaceutical compositions comprising PDE inhibitors which may be used in the method of the invention include all compositions wherein the PDE inhibiotrs are contained in an amount effective to achieve the intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Suitable pharmaceutically acceptable vehicles are well known in the art and are described for example in Gennaro et al, 2000, [**Gennaro et al. 2000**, of *Remington's Pharmaceutical Sciences*, Part 8, 20th Edition, Merck Publishing Company, Easton, Pennsylvania] a standard reference text in this field. Pharmaceutically acceptable vehicles can be routinely selected in accordance with the mode of administration and the solubility and stability of the PDE inhibitors. For example, formulations for intravenous administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. The use of biomaterials and other polymers for drug delivery, as well the different techniques and models to validate a specific mode of administration, are disclosed in the literature (Luo et al. 2001, Exp Opin Ther Patents, 11: 1395-1410; Cleland et al., Curr Opin Biotechnol, 12: 212-9, 2001).

The PDE inhibitors may be administered by any means that achieves the intended purpose. For example, administration may be by a number of different routes including, but not limited to subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intra-cerebral, intrathecal, intranasal, oral, rectal, transdermal, intranasal or buccal. Preferably the PDE inhibitors are administered orally.

Parenteral administration can be by bolus injection or by gradual perfusion over time. It is understood that the dosage administered will be dependent upon the age, health, and weight of the recipient, concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The total dose required for each treatment may be administered by multiple doses or in a single dose.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, a suspension of the active compound as an oily injectable formulation may be administered.

Depending on the intended route of delivery, the PDE inhibitors may be formulated as injectable or oral compositions. The compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a pre-determined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include pre-filled, pre-measured ampoules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the PDE inhibitor is usually a minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

Liquid forms suitable for oral administration may include a suitable aqueous or non-aqueous vehicle with buffers, suspending and dispensing agents, colorants, flavours and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatine; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavouring agent such as peppermint, methyl salicylate, or orange flavouring.

Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art.

The above-described components for orally administered or injectable compositions are merely representative. Further materials as well as processing techniques and the like are known to the skilled practitioner *(Gennaro et al., 2000).*

PDE inhibitors can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials is also known to the skilled practitioner (Karsa et al. 1993, (Ed), Encapsulation and Controlled Release; Stephenson (Editor); Springer Verlag; Yacobi et al. 1998, Oral Sustained Release Formulations: Design and Evaluation, Eva Halperin-Walega (Editor), 1st Ed. edition; Pergamon Press).

By "effective amount", is meant an mount sufficient to achieve a concentration of PDE inhibitor which is capable of promoting follicular growth, with or without exogenous FSH or FSH replacements. Such concentrations can be routinely determined by those of skill in the art. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the patient's endogenous FSH levels, and the like.

The expression "Pharmaceutically acceptable" is meant to encompass any carrier which does not substantially interfere with the effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which is administered. For example, for parenteral administration, the above active ingredients may be formulated in unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

Besides the pharmaceutically acceptable carrier, compositions comprising PDE inhibitors can also comprise minor amounts of additives, such as stabilizers, excipients, buffers and preservatives.

Examples

MATERIALS AND METHODS

Animals

Immature Sprague Dawley CD (SD) BR female rats (Charles River, Calco, Italy), weighing 36-39 g on receipt were used. The animals were housed in a room under the following constant environmental conditions: temperature 22°C ± 2, relative humidity 55% ± 10, 15-20 air changes per hour (filtered on HEPA 99.99%) and artificial light with a 12-hour circadian cycle (7h00 – 19h00).

For the entire duration of the study the rats were kept in wire cages (cm. 40.5x38.5x18h) with stainless steel feeders and fed on a standard pelleted diet (4RF21 produced by Charles River Italia's licensee Mucedola s.r.l.) and filtered water "ad libitum".

Materials

Human recombinant follicle stimulating hormone (r-hFSH) and human recombinant chorionic gonadotrophin (r-hCG) were supplied by Laboratoires Serono Aubonne (LSA, Aubonne, Switzerland). Test Compounds (Dipyridamole, Zaprinast, Sildenafil) were either synthesized based on published compound synthesis methods or purchased from commercial sources.

Experimental Procedure

Immature female rats arrived on Friday of the week prior to the experimentation at 18-19 days of age along with a lactating female (ten pups per lactating female). All rats were weaned from the mother on the following Monday (21-22 days old) and were randomly sorted into the experimental groups (6-8 animals/group).

The rats were subcutaneously injected (in the scruff of the neck) twice a day for two days (first injection: 8.30 – 9.00 and second injection 15.30 – 16.00) with r-hFSH or vehicle (PBS) at a volume of 250 µL/injection. The doses of FSH injected were either suboptimal (606 ng/rat total dose split over four injections; indicated as 'Low FSH' in Figures) or high (2424.8 ng/rat total dose split over 4 injections; indicated as 'High FSH' in Figures) as a positive control.

In addition to the above injections, rats were also injected subcutaneously with test compounds or vehicle twice a day for 2 days at indicated doses (mg/kg/injection), concomitantly with the FSH injections. These compounds were diluted in either NP3S (n-methyl-2-pyrrolidone 5%, polyethylene glycol 400 30%, polyethylene glycol 200 25%, propylene glycol 20%, saline 20%) or aqueous vehicles. Therefore, the total number of injections received by each rat to promote follicle growth was eight, including four injections of r-hFSH or r-hFSH vehicle plus four injections of test compound or test compound vehicle.

On day 2 of the experiment and along with the final r-hFSH injection, the rats were also treated with a single subcutaneous injection of r-hCG (1430 ng/rat) to induce ovulation of all or most of the matured follicles.

At 10h00 of the morning following r-hCG administration, rats were euthanized by CO_2 asphyxia. The animals were laid on their backs and undersides were sprayed with ethanol to both sterilize and keep the hair from falling out in the dissection of the animals. With the aid of scissors and forceps the skin and muscle were cut starting from the pubic symphisis with aboral-oral direction up to the sternum. The internal organs were exposed and the intestine was moved to one side. The ovaries, the uterine horns and the uterus body were removed clipping away the fat and the connective tissue. The

entire reproductive tract was then placed into a well in a 24 well plate containing PBS (1 animal/well).

After all the animals were sacrificed and the ovaries were harvested, the oviducts were gently removed from the ovaries, dipped in PBS and placed on a microscope slide. The ovary was then taken out, cleaned and placed into PBS for weighing (the uterus was discarded).

Pairs of oviducts were placed on one slide and then a slide was placed on top of the first slide using a piece of tape to secure the frosted ends of the slides together. After the oviducts were placed on the bottom slide, the top slide was folded over and the non-frosted end was then taped, compressing the oviducts between the two slides. The oviducts were then examined by a light microscope under contrast phase conditions (at a minimum of 40× magnification) and the ova, if any, present in the two ampullae for each rat were counted. The ovaries were dried on a paper towel and weighed by placing the two ovaries from each animal in a weight boat on a tared balance.

In one experiment, ovaries were harvested from rats at midday of the second day of treatment for histological analysis, prior to the last set of injections. These rats received only the first three doses of FSH and test compound prior to euthanasia and organ harvest. The secondary follicles were evaluated by counting the total number of secondary follicles: including both small follicles (those at any intermediate stage of maturation, having a multilayered granulosa with the first, scattered vacuoles, but without an antrum) and antral follicles (those having antral dilation, with an external diameter of around 500 microns, or higher, with or without thinning of the granulosa cell layer). The antral follicles (≥ 500 mcm) were also counted separately.

Data Analysis

The proportion of ovulating animals, the average number ova present in the ampulla per rat, and the average ovary weight were calculated from the single values in each experimental group and the relevant graphs were plotted. All Figures demonstrate mean plus/minus standard error of the mean. In the Figures, the number above the standard error bars indicates the number of rats in the group that had one or more ovulated ova

compared to the total number of rats in the group, for example X/Y means that X rats out of a total of Y had one or more ovulated oocytes.

Compounds tested

The following compounds were tested in the above protocols:
Zaprinast (inhibitor of PDE's 1, 5, 6, 7, 9, 10, 11);
Sildenafil (inhibitor of PDE's 1, 5 and 6);
Dipyridamole (Inhibitor of PDE's 5, 6, 7, 8, 10, 11);

These molecules have been shown to exhibit the following selectivity for various PDE enzymes:

Molecule	IC_{50} PDE1	IC_{50} PDE5	IC_{50} PDE6	IC_{50} PDE3 [*]
Zaprinast	9400nM [ref 1]	2000nM [ref 1]		>100,000nM [ref 1]
Sildenafil	260 nM [ref 1]	3.0-3.6 nM [ref 1]	Selectivity for PDE5 10-fold > PDE6 [ref 2]	65,000 nM [ref 1]

ref 1: Terrett-NK, Bell AS, Brown D, and Ellis P. (1996) Sildenafil (Viagra TM), a potent and selective inhibitor of type 5 CGMP phosphodiesterase with utility for the treatment of male erectile dysfunction, Biooganic and Medicinal Chemistry Letters, 6(15):1819-1824.
ref 2: Physicians Desk Reference, 57th edition, 2003

*PDE3 is expressed in oocytes, preferred PDE inhibitors are selective against PDE3, because inhibition of PDE3 is detrimental to the oocytes[3].

Results

Dipyridamole (an inhibitor of PDE's 5, 6, 7, 8, 10, 11) was administered at doses of 1, 5 and 25 mg/kg x 4 injections per rat (subcutaneously) in NP3S with the Low Dose of FSH, resulting in an increase in the number of ovulated oocytes per rat as compared to the Low Dose of FSH plus NP3S vehicle control. With the FSH low dose alone, an average of one oocyte per rat was collected, and only 4 out of ten rats ovulated. In contrast, when the FSH low dose was in conjunction with Dipyrimadole (1, 5 or 25 mg/kg) the average number of oocytes per rat was 7.5, 6.8 and 6.2, respectively, and 7 out of 10, 6 out of 10 or 10 out of 10 rats ovulated, respectively. These results are shown in Figure 1.

Zaprinast (an inhibitor of PDE's 1, 5, 6, 7, 9, 10, 11) was administered at doses of 1, 5 and 25 mg/kg x 4 injections per rat (subcutaneously) in NP3S with the Low Dose FSH, resulting in a dose-related increase in the number of ovulated oocytes per rat as compared to the Low Dose FSH plus NP3S vehicle control. With the FSH low dose alone, an average of 2.4 oocytes per rat was collected, and only 5 out of ten rats ovulated (Experiment 1). In contrast, when the FSH low dose was in conjunction with Zaprinast (1, 5 or 25 mg/kg) the average number of oocytes per rat was 3.25, 6.4, 9.5, respectively, and 6 out of 10, 9 out of 10 or 10 out of 10 rats ovulated (Experiment 1), respectively. These results are shown in Figure 2.

Sildenafil (an inhibitor of PDE's 1, 5 and 6) was administered at doses of 1, 5 and 25 mg/kg x 4 injections per rat (subcutaneously) in NP3S with the Low Dose FSH, resulting in a dose-related increase in the number of ovulated oocytes per rat as compared to the Low Dose FSH plus NP3S vehicle control. With the FSH low dose alone, an average of 1 oocyte per rat was collected, and only 3 out of ten rats ovulated (Experiment 1). In contrast, when the FSH low dose was in conjunction with Sildenafil (1, 5 or 25 mg/kg) the average number of oocytes per rat was 3.5, 5.5, 6.9, respectively, and 6 out of 8, 5 out of 8 or 7 out of 8 rats ovulated (Experiment 1), respectively. These results are shown in Figure 3.

The total number of secondary follicles per rat (2 ovaries) in rats treated with vehicle, Low FSH, High FSH, or Low FSH + 75 mg/kg Sildenafil is shown in Figure 4. The total number of secondary follicles is higher in the Sildenafil + FSH group than in any of the other groups [Vehicle=69; Low FSH=64; Low FSH plus Sildenafil=84; High FSH=64].

The total number of antral follicles per rat (2 ovaries) in rats treated with vehicle, Low FSH, High FSH, or Low FSH + 75 mg/kg Sildenafil, is shown in Figure 5. The total number of antral follicles in rats treated with Sildenafil + FSH is higher than the number of antral follicles in rats treated with Low FSH [Vehicle=3.2; Low FSH=4.1; Low FSH plus Sildenafil=7.4; high FSH=9.5].

Sildenafil was administered at doses of 1, 5 and 25 mg/kg x 4 injections per rat (subcutaneously) in aqueous vehicle with the Low Dose FSH, resulting in a dose-related

increase in the number of ovulated oocytes per rat as compared to the Low Dose FSH alone plus aqueous vehicle control. With the FSH low dose alone, an average of 1 oocyte per rat was collected, and only 2 out of 8 rats ovulated. In contrast, when the FSH low dose was in conjunction with Sildenafil (5 mg/kg) the average number of oocytes per rat was 20, and 7 out of 8 rats ovulated. These results are shown in Figure 6. [Note: Fig 6 differs from Fig 3 in that the vehicle is aqueous rather than organic NP3S-SM]

Other PDE inhibitors were tested in the above model, and the results are listed in Table 1.

Table 1. Follicular growth activity of various PDE inhibitors

Compound	PDE selectivity	Follicular growth activity with Low FSH?
Papavarine	Non-selective	NO
Sildenafil	PDE1, 5, 6	YES
Ariflo	PDE4	Inhibits follicular growth
Dipyridamole	PDE5, 6, 7, 8, 10, 11	YES
Zaprinast	PDE1, 5, 6, 7, 9, 10, 11	YES
CDP840	PDE4	Inhibits follicular growth

References:

[1] Healy *et al.*; *Lancet* **343** *1994*; 1539-1544

[2] Klein *et al.; Pharmacokinetics and pharmacodynamics of single-chain recombinant human follicle-stimulating hormone containing the human chorionic gonadotrophin carboxyterminal peptide in the rhesus monkey*; *Fertility & Sterility*; **2002**, *77*, 1248-1255

[3] A Wiersma, B Hirsch, A Tsafriri, R Hanssen, M Van de Kant, H Kloosterboer, M Conti, A Hsueh. *Phosphodiesterase 3 inhibitors suppress oocyte maturation and consequent pregnancy without affecting ovulation and cyclicity in rodents; J. Clin. Invest.*; **1998**, *102*(3): 532-537.

Claims

1. Use of an inhibitor of a PDE enzyme for the preparation of a medicament for stimulating ovarian follicular growth in a patient.

2. Use according to claim 1, wherein the patient is undergoing ovulation induction.

3. Use according to claim 1 or 2, wherein the patient is undergoing controlled ovarian hyperstimulation for assisted reproductive technologies.

4. Use according to claim 1, 2 or 3, wherein the medicament is for simultaneous, separate or sequential administration with FSH, or an agent having FSH activity, or an agent leading to endogenous FSH release.

5. Use according to claim 1, 2 or 3, wherein the medicament is for simultaneous, separate or sequential administration with FSH.

6. Use according to claim 1, 2 or 3, wherein the medicament is for simultaneous, separate or sequential administration with an agent having FSH activity, or an agent leading to endogenous FSH release.

7. Use according to any one preceding claim, wherein the medicament is administered starting at or about day 2 to 3 after menses.

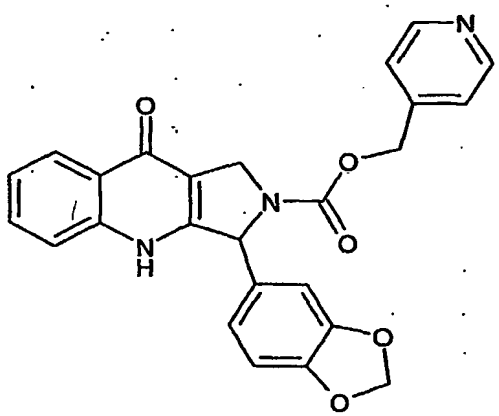
8. Use according to any one preceding claim, wherein the medicament is administered on a daily basis until follicular growth is sufficient, at which point an ovulation triggering dose of hCG, preferably 5'000-10'000 IU; is administered.

9. Use according to any one preceding claim, wherein the medicament is administered with FSH, and wherein the dose of FSH is reduced with respect to the dose required in the same patient in the absence of the PDE inhibitor, in order to achieve the same result in terms of follicular growth.

10. Use according to any one preceding claim, wherein the PDE inhibitor is an inhibitor of at least one PDE type selected from 1, 5 and 6.

11. Use according to any one preceding claim, wherein the PDE inhibitor is selected from: 5-[2-ethoxy-5-(4-methyl-1 -piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil); Zaprinast; dipyrimadole; 5-(2-ethoxy-5-morpholinoacetylphenyl)-1 -methyl-3-n-propyl-1,6-dihydro-7H-20 pyrazolo[4,3-d]pyrimidin-7-one; 3-ethyl-5-[5-(4-ethylpiperazin- 1 -ylsulphonyl)-2-n-propoxyphenyl]-2-(pyr- idin-2-yl) methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridi- n-3-yl]-2-(pyridin-2-yl) methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; (+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7 H-pyrazolo[4 ,3-d] pyrimidin-7-one; 5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-[2-iso-butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-[2-ethoxy-5-(4-ethylpiperazin-1 -ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-(5-acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1 -isopropyl-3-azetidinyl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one; 5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2 ,6-dihydro-7H-pyrazolo [4,3-d]pyrimidin-7-one; (6R, 12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(-3,4-methylenedioxyphenyl)pyrazino[2',1 ':6,1]pyrido[3,4-b]indole-1,4-dione (Tadalafil; IC-351), the compound of examples 78 and 95 of published international application W0 95/19978, as well as the compound of examples 1, 3, 7 and 8 therein; 2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo [5,1 -f][1,2,4]triazin-4-one (vardenafil); the compound of example 11 of published international application W093/07124 (EISAI); compounds 3 and 14 from Rotella D P, J. Med. Chem., 2000, 43,1257; 4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2H)pyridazinone; 1 -[4-[(1,3-benzodioxol-5-ylmethyl)amiono]-6-chloro-2-quinozolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-cis-5,6a,7,9,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-[4,5]imidazo[2,1-b]purin-4(3H)one; furaziocillin; cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a-octahydrocyclopent[4,5]-imidazo[2- ,1 -b]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6- carboxylate; 3-acetyl-1 -(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl)propoxy)-3-(2H) pyridazinone; 1-methyl-5(5-

morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro- 7H-pyrazolo (4,3-d)pyrimidin-7-one; 1 -[4-[(1,3-benzodioxol-5-yl methyl)amino]-6-chloro-2-quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt; Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; see WO 96/26940); Pharmaprojects No. 5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 and E-4010 (Eisai); Bay-38-3045 & Bay-38-9456 (Bayer), Vinpocetine (Richter Gideon); SCH-51866 (Schering-Plough), SCH-59498, compounds no. 31, 33, 50 described in Ahn *et al.*; *J. Med. Chem.*; **1997**, *40*, 2196-2210, dipyridamole, AWD-12-171 and AWD-12-217 (ASTA Medica), BMS-341400 (Bristol Meyers Squibb), UK-343,664 (Pfizer), 5E-3623, 5E-3569, 5E-3657, E4021 (Eisai), KS-505a (Kyowa Hakko Kogyo), YC-1 (Yung Shin Pharmaceutical Industries), IDDB reference number 323951 (Bayer), WIN-61691 (Sanofi Winthrop), FR226807 (Fujisawa), IDDB references 461317, 462503, 461321, 461324, 466146 (Johnson & Johnson),



compounds listed in Table 1 of Jiang *et al.*; *J. Med. Chem.*; **2003**, *46*, 441-444, particularly compounds 20b, 20e, 20f, 20l, 20o, 20p, (-)-20q, 20t, 20u, 20v, 20w and 26a.

12. Use according to any one preceding claim, wherein the PDE inhibitor is selected from Sildenafil and Zaprinast, Dipyrimidole, and compounds no. 31 and 33, described in Ahn *et al.*; *J. Med. Chem.*; **1997**, *40*, 2196-2210.

13. Use according to any one of claims 1 to 10, wherein the PDE inhibitor is a selective PDE 1 inhibitor.

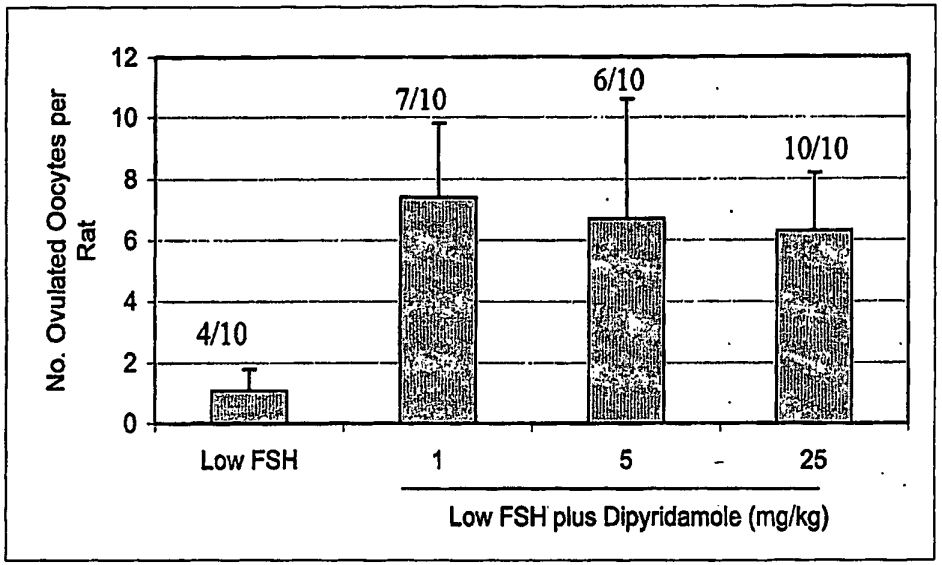
14. Use according to any one of claims 1 to 10, wherein the PDE inhibitor is Zaprinast.

15. Use according to any one of claims 1 to 10, wherein the PDE inhibitor is Sildenafil.

Abstract

Disclosed is the use of PDE inhibitors in promoting ovarian follicular growth.

Figure 1


No. Ovulated Oocytes per Rat
12
10
8
6
4
2
0
4/10
7/10
6/10
10/10
Low FSH
1
5
25
Low FSH plus Dipyridamole (mg/kg)

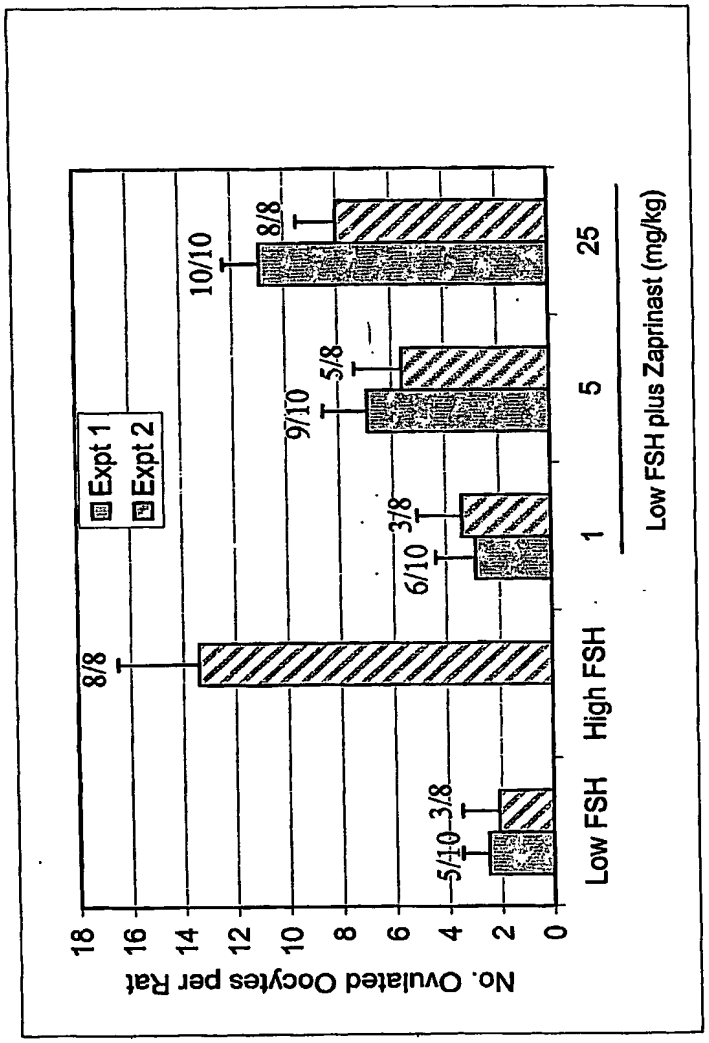


Figure 2

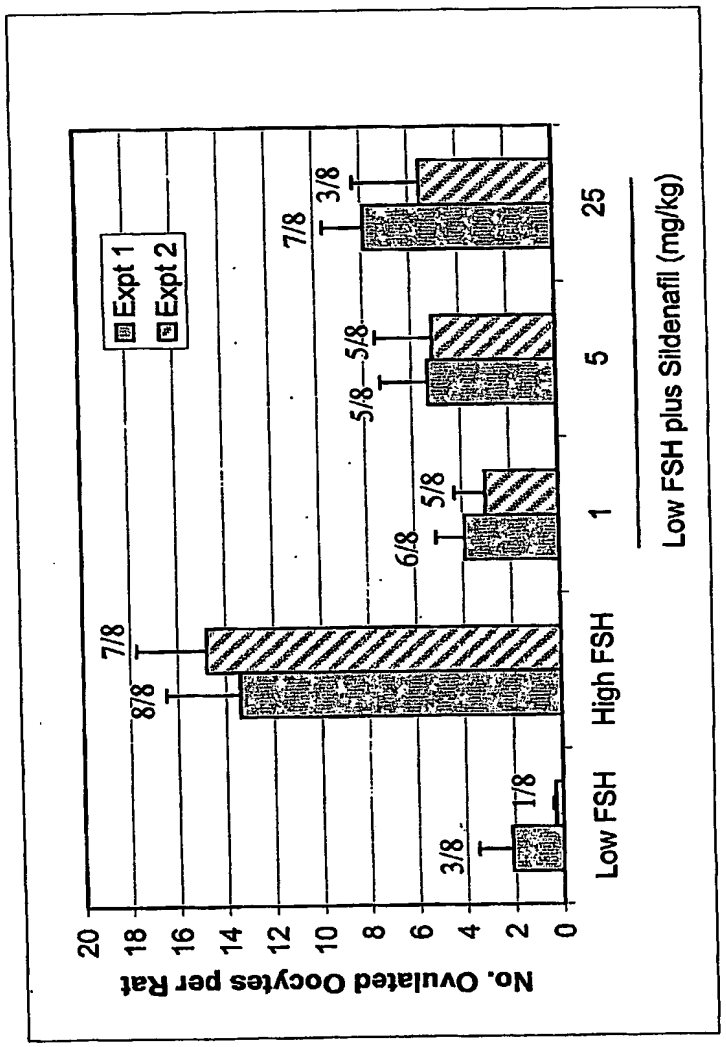


Figure 3

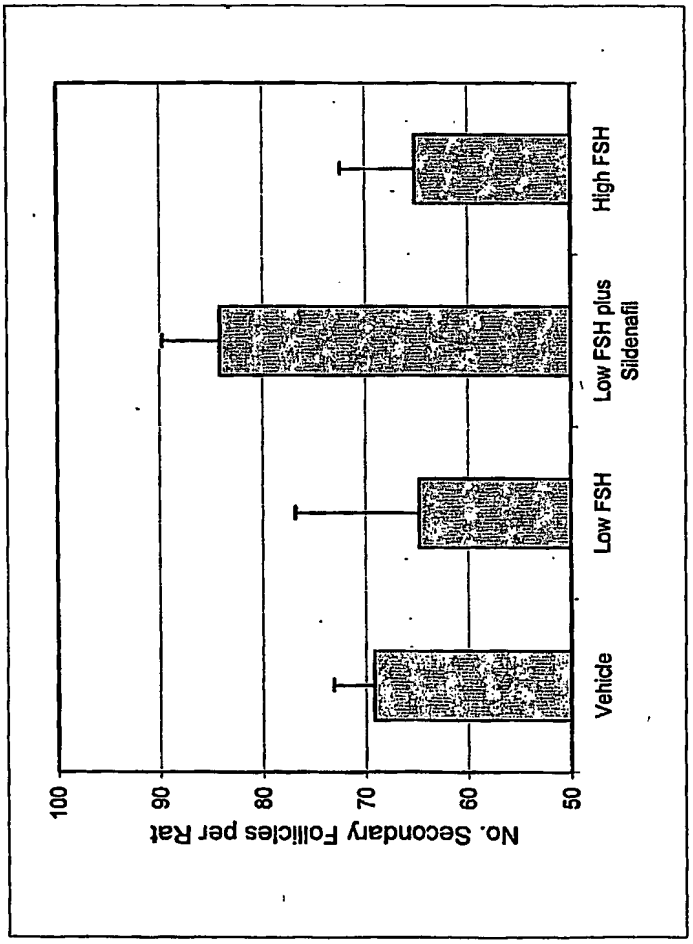


Figure 4

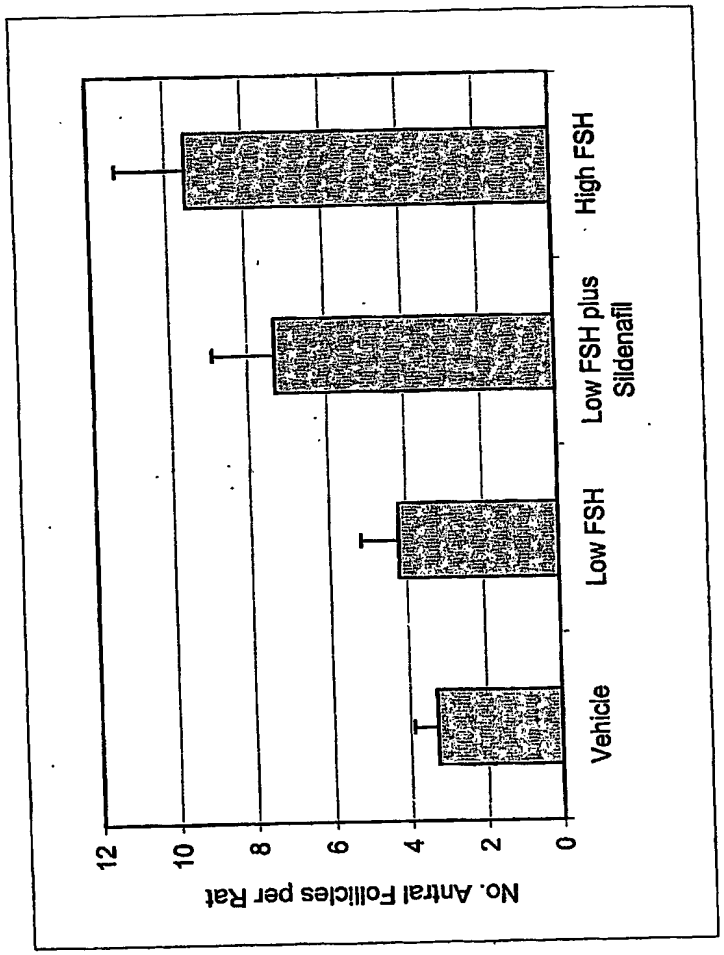
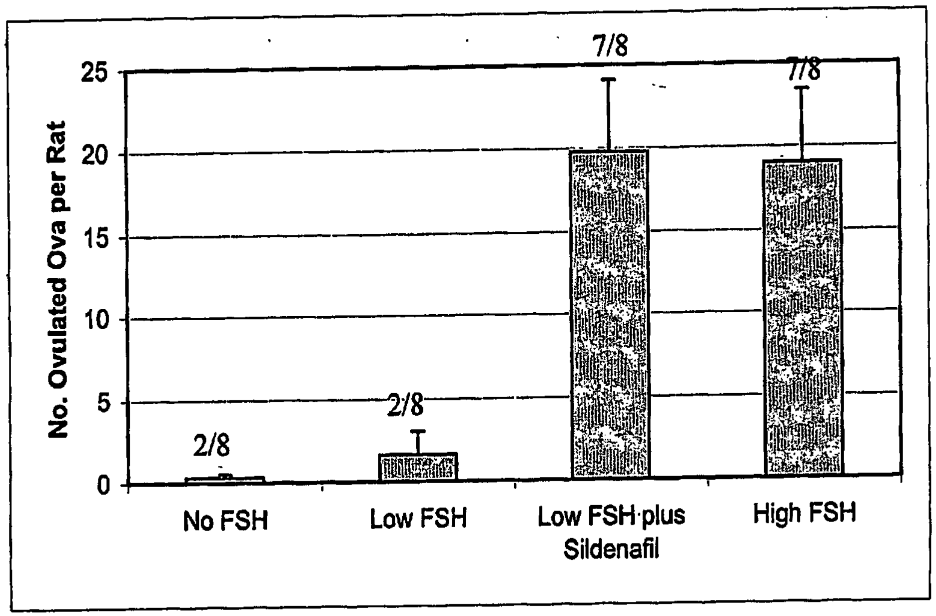


Figure 5

Figure 6



No. Ovulated Ova per Rat
25
20
15
10
5
0
2/8
2/8
7/8
7/8
No FSH
Low FSH
Low FSH plus Sildenafil
High FSH